

CLAIMS

We claim:

- 5 1. A substantially purified salivary *P. ariasi* polypeptide.
2. The polypeptide of claim 1, wherein the polypeptide comprises
- a) an amino acid sequence at least 80% identical to a the amino acid
sequence set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7,
10 SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17,
SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27,
SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37,
SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, or SEQ ID
NO:47,
- 15 b) a conservative variant of the amino acid sequence set forth as SEQ ID
NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11,
SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21,
SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31,
SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41,
20 SEQ ID NO:43, SEQ ID NO:45, or SEQ ID NO:47
- c) an immunogenic fragment comprising eight consecutive amino acids of
the amino acid sequence set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5,
SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15,
SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25,
25 SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35,
SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45,
or SEQ ID NO:47, that specifically binds to an antibody that specifically binds the
amino acid sequence set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ
ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID
30 NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID
NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID

-113-

NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, or SEQ ID NO:47, respectively; or

d) the amino acid sequence set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, or SEQ ID NO:47, wherein administration of the polypeptide to a subject produces an immune response to *P. ariasi*.

10

3. The *P. ariasi* polypeptide of claim 2, wherein the polypeptide comprises an amino acid sequence as set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, or SEQ ID NO:47, or a conservative variant thereof.

4. The *P. ariasi* polypeptide of claim 3, wherein the polypeptide comprises an amino acid sequence set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, or SEQ ID NO:47.

5. An antigenic fragment of the polypeptide of claim 4.

6. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence at least 80% identical to an amino acid sequence set forth as SEQ ID NO:11, SEQ ID NO: 19, SEQ ID NO:35, or SEQ ID NO: 39.

-114-

7. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:33, SEQ ID
5 NO:39, or SEQ ID NO: 45.

8. An isolated nucleic acid encoding the polypeptide of claim 1.

9. The nucleic acid of claim 8, wherein the nucleic acid comprises a
10 sequence as set forth as SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, or SEQ
15 ID NO:48, or a degenerate variant thereof.

10. The nucleic acid of claim 8, wherein the nucleic acid comprises a sequence as set forth as SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, or SEQ
20 ID NO:48.

25 11. The nucleic acid of claim 8, wherein the nucleic acid encodes an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:12, SEQ ID NO: 20, SEQ ID NO:36, or SEQ ID NO: 40.

12. The nucleic acid of claim 8, wherein the nucleic acid encodes an amino
30 acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID

-115-

NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:34, SEQ ID NO:40, or SEQ ID NO: 46.

13. The nucleic acid of claim 8, operably linked to an expression control
5 sequence.

14. The nucleic acid of claim 13, wherein the expression control sequence comprises a promoter.

10 15. The nucleic acid of claim 14, wherein the promoter comprises an inducible or constitutive promoter.

16. The nucleic acid of claim 15, wherein the promoter comprises a cytomegalovirus promoter.

15

17. A vector comprising the nucleic acid of claim 8.

18. The vector of claim 17, wherein the vector comprises a plasmid.

20 19. The vector of claim 17, wherein the vector comprises a viral vector.

20. A host cell transformed with the vector of claim 17.

21. An antibody that specifically binds the polypeptide of claim 1.

25

22. The antibody of claim 21, wherein the antibody comprises a monoclonal antibody.

23. The antibody of claim 21, comprising a detectable label.

30

24. The antibody of claim 23, wherein the label comprises a fluorescent, enzymatic or radioactive label.

-116-

25. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

5 26. A pharmaceutical composition comprising the nucleic acid of claim 8 and a pharmaceutically acceptable carrier.

27. A method for inducing an immune response to a *P. ariasi* polypeptide in
10 a subject, comprising
administering to the subject a therapeutically effective amount of the *P. ariasi* polypeptide of claim 1, or a polynucleotide encoding the polypeptide the *P. ariasi* polypeptide of claim 1, thereby inducing the immune response.

15 28. The method of claim 27, wherein the immune response comprises a T cell response.

29. The method of claim 27, wherein the immune response comprises a B cell response.

20 30. The method of claim 27, wherein the subject comprises a non-human veterinary subject.

31. The method of claim 27, wherein the subject is a dog.

25 32. The method of claim 23, wherein the subject is a human.

33. The method of claim 27, wherein the polypeptide comprises
an amino acid sequence at least 80% identical to a the amino acid sequence set forth
30 as SEQ ID NO:11, SEQ ID NO: 19, SEQ ID NO:35, or SEQ ID NO: 39.

34. The method of claim 27, wherein the polypeptide comprises an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ

-117-

ID NO:1, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:33, SEQ ID NO:39, or SEQ ID NO: 45.

5 35. A method for inhibiting a symptom of a *Leishmania* infection or preventing a *Leishmania* infection in a subject, comprising administering to the subject a therapeutically effective amount of the *P. ariasi* polypeptide of claim 1, or a polynucleotide encoding the polypeptide, thereby inhibiting the symptom of the *Leishmania* infection or preventing the *Leishmania* infection.

10

36. The method of claim 35, wherein the polypeptide comprises an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:11, SEQ ID NO: 19, SEQ ID NO:35, or SEQ ID NO: 39.

15

37. The method of claim 35, wherein the polypeptide comprises an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:33, SEQ ID NO:39, or SEQ ID NO: 45.

20

38. Use of a composition comprising the polypeptide of claim 1 or a nucleic acid encoding the polypeptide of claim 1 for the manufacture of a medicament.

39. A substantially purified salivary *P. perniciosus* polypeptide.

25

40. The polypeptide of claim 39, wherein the polypeptide comprises

a) an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83;

30

-118-

b) a conservative variant of the amino acid sequence set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83;

c) an immunogenic fragment comprising eight consecutive amino acids of the amino acid sequence set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83, that specifically binds to an antibody that specifically binds the amino acid sequence set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83, respectively; or

d) the amino acid sequence set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83, wherein administration of the polypeptide to a subject produces an immune response to *P. perniciosus*.

41. The *P. perniciosus* polypeptide of claim 40, wherein the polypeptide comprises an amino acid sequence as set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83, or a conservative variant thereof.

30

42. The *P. perniciosus* polypeptide of claim 41, wherein the polypeptide comprises an amino acid sequence set forth as SEQ ID NO:49, SEQ ID NO:51, SEQ

-119-

ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, or SEQ ID NO:83.

5

43. An antigenic fragment of the polypeptide of claim 42.

44. The polypeptide of claim 39, wherein the polypeptide comprises an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:55, SEQ ID NO: 63, SEQ ID NO:73, or SEQ ID NO: 75.

10

45. The polypeptide of claim 39, wherein the polypeptide comprises an amino acid sequence at least 80% identical to an amino acid sequence set forth as SEQ ID NO:73 or SEQ ID NO: 75.

15

46. An isolated nucleic acid encoding the polypeptide of claim 39.

47. The nucleic acid of claim 46, wherein the nucleic acid comprises a sequence as set forth as SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, or SEQ ID NO:84, or a degenerate variant thereof.

20

48. The nucleic acid of claim 46, wherein the nucleic acid comprises a sequence as set forth as SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, or SEQ ID NO:84.

25

30

-120-

49. The nucleic acid of claim 46, wherein the nucleic acid encodes an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:56, SEQ ID NO: 64, SEQ ID NO:74, or SEQ ID NO:76.

5 50. The nucleic acid of claim 46, wherein the nucleic acid encodes an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:74 or SEQ ID NO: 76.

10 51. The nucleic acid of claim 46, operably linked to an expression control sequence.

52. The nucleic acid of claim 51, wherein the expression control sequence comprises a promoter.

15 53. The nucleic acid of claim 52, wherein the promoter comprises an inducible or constitutive promoter.

20 54. The nucleic acid of claim 52, wherein the promoter comprises a cytomegalovirus promoter.

55. A vector comprising the nucleic acid of claim 46.

56. The vector of claim 55, wherein the vector comprises a plasmid.

25 57. The vector of claim 55, wherein the vector comprises a viral vector.

58. A host cell transformed with the vector of claim 55.

30 59. An antibody that specifically binds the polypeptide of claim 39.

60. The antibody of claim 59, wherein the antibody comprises a monoclonal antibody.

-121-

61. The antibody of claim 59, comprising a detectable label.

62. The antibody of claim 61, wherein the label comprises a fluorescent,
5 enzymatic, or radioactive label.

63. A pharmaceutical composition comprising the polypeptide of claim 39
and a pharmaceutically acceptable carrier.

10 64. A pharmaceutical composition comprising the nucleic acid of claim 46
and a pharmaceutically acceptable carrier.

65. A method for inducing an immune response to a *P. perniciosus*
polypeptide in a subject, comprising
15 administering to the subject a therapeutically effective amount of the *P.*
perniciosus polypeptide of claim 39, or a polynucleotide encoding the polypeptide
the *P. perniciosus* polypeptide of claim 39, thereby inducing the immune response.

66. The method of claim 65, wherein the immune response comprises a T
20 cell response.

67. The method of claim 65, wherein the immune response comprises a B
cell response.

25 68. The method of claim 65, wherein the subject comprises a non-human
veterinary subject.

69. The method of claim 65, wherein the subject is a dog.

30 70. The method of claim 65, wherein the subject is a human.

-122-

71. The method of claim 65, wherein the polypeptide comprises an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:55, SEQ ID NO: 63, SEQ ID NO:73, or SEQ ID NO: 75.

5 72. The method of claim 65, wherein the polypeptide comprises an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:73 or SEQ ID NO: 75.

73. A method for inhibiting a symptom of *Leishmania* or preventing
10 *Leishmania* infection in a subject comprising administering to the subject a therapeutically effective amount of the *P. perniciosus* polypeptide of claim 39, or a polynucleotide encoding the polypeptide, thereby inhibiting the symptom of *Leishmania* or preventing the *Leishmania* infection.

15 74. The method of claim 73, wherein the polypeptide comprises an amino acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:55, SEQ ID NO: 63, SEQ ID NO:73, or SEQ ID NO: 75.

75. The method of claim 73, wherein the polypeptide comprises an amino
20 acid sequence at least 80% identical to a the amino acid sequence set forth as SEQ ID NO:73 or SEQ ID NO: 75.

76. Use of a composition comprising the polypeptide of claim 39 or a
nucleic acid encoding the polypeptide of claim 39 for the manufacture of a
25 medicament.